Service Priorities and Programmes

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Implementation of PLA2R testing on renal biopsy for membranous glomerulonephritis - using a de novo readily available antigen retrieval method for formalin-fixed paraffin embedded sections

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Introduction

M-type phospholipase A2 receptor (PLA2R) was identified to be a major antigen of autoantibodies that were detected in 70% of primary membranous glomerulonephritis (MGN) patients but were less common or absent in patients having secondary MGN. Either detection of serum autoantibodies for PLA2R (anti-PLA2R test) or staining for overexpression of PLA2R in the glomeruli in renal biopsy by immunofluorescence study (IMF) or immunohistochemical study can be performed. Many previously reported literatures tested PLA2R by IMF on formalin-fixed paraffin-embedded sections (FFPE), however, the antigen retrieval methods described (AgRM), such as pronase-based and proteinase K-based methods, are labour intensive and space demanding, and appeared to yield a non-uniform glomerular staining performance.

Objectives

To develop a practical, economical and reliable technical method to implement PLA2R testing on renal biopsy FFPE.

Methodology

We developed a de novo method to achieve the AgRM for FFPE, using a currently and commonly available Bond autoimmunostainer and the readily available antigen retrieval reagents (ER2 15 minutes + Protease 20 minutes). Sigma-Alrich rabbit polyclonal antiPLA2R antibodies (1:100) and secondary antibody ThremoFisher Alexa Fluor 488 – conjugate Goat antiRab (1:100) were applied. The slides, original renal biopsy pathological findings and clinical features of the cases were reviewed by a renal pathologist.

Result

Based on this technical method, 23 cases were tested. PLA2R test achieved a sensitivity of 71% for non-stage I primary MGN cases, a sensitivity of 50% for clinically primary MGN cases with some subtle unusual pathological features, and a specificity of 100% for secondary MGN cases. All cases showed uniform strong diffuse global

granular glomerular staining pattern. The overall performance is comparable to the results reported in literature. This a de novo AgRM on FFPE for IMF which has dramatically simplified the labour intensive steps and is practical to be applied in many anatomical pathology laboratories. Potentially various IMF tests could be attempted using FFPE, with the AgRM described, when fresh tissue is not available. The result of PLA2R test also currently serves as one of the parameters for consideration of clinical management for MGN patients.